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PREPARATION OF FLUORESCEIN-LABELLED IMMUNE GLOBULIN FOR THE IDENTIFICATION OF *Treponema pallidum**†

BY

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The dye compound fluorescein isothiocyanate conjugated with *Treponema pallidum* antibodies has been used in various ways in the study of syphilis. Techniques for the identification of *T. pallidum* which utilize direct fluorescent antibody reactions in human and experimental-rabbit tissues are both practical and relatively simple to perform (Yobs, Brown, and Hunter, 1964; Yobs, Olansky, Rockwell, and Clark, 1965; Yobs, Rockwell, and Clark, 1964; Yobs, Clark, Mothershed, Bullard, and Artley, 1968). A rapid immunofluorescent stain (RIS) technique (Kellogg and Deacon, 1964) for the identification of *T. pallidum* in human syphilitic lesions was described and the fluorescent antibody darkfield (FADF) method (Kellogg and Deacon, 1965) was subsequently defined. This last technique was the combination of two previously reported methods; namely, the RIS test performed in conjunction with a human anti-*T. pallidum* serum conjugated with fluorescein isothiocyanate and absorbed with Reiter treponemes to remove the "common" group antigen (Deacon and Hunter, 1962). Treponemal antibody production and absorption techniques that utilize various treponemes have shown that individual treponemes can be identified effectively, as well as classified immunologically (Deacon and Hunter, 1962; Meyer and Hunter, 1967).

This paper describes the preparation of a better defined, more specific rabbit anti-*T. pallidum* fluorescein-conjugated globulin. In general, sequential bleedings are used to ascertain the optimal time for the harvesting of the antiserum, and absorption and dilution techniques are used to narrow the specificity of the antiserum.

Material and Methods

Immune Sera Seven adult (3–4 kg.) male rabbits were selected on the basis of well-developed testes and lack of clinical evidence of disease. They were tested by five serological tests: *Treponema pallidum* immobilization (TPI); VDRL slide quantitive (VDRL); Kolmer Reiter protein (KRP) (U.S. Department of Health, Education, and Welfare, 1964); fluorescent treponemal antibody-absorption (FTA-ABS) (Provisional Technique, 1965); and fluorescent treponemal antibody (FTA-1:5) (Mothershed, Yobs, and Clark, 1967). All the test results were non-reactive. The rabbits were observed for an additional month and then re-tested by the same five tests. Five of the seven rabbits were seronegative in all tests. Two rabbits with weakly reactive results to the KRP test were dropped from the experiment. Approximately 40 ml. blood were drawn from each rabbit, and the serum was frozen at −20°C. for the preparation of a pre-inoculation fluorescein-labelled "normal rabbit" globulin conjugate. Each rabbit was then inoculated intratesticularly with $2{\cdot}5 \times 10^7$ *T. pallidum* into each testicle. The rabbits were maintained in individual cages and bled arbitrarily at 3, 6, 16, and 26 weeks after inoculation. Clinical orchitis was present in all rabbits by day 4, and treponemes could be demonstrated by darkfield examination of testicular aspirates. Serological tests were performed at each bleeding on individual sera and on the pooled sera. The pooled sera consisted of equal amounts of serum from the individual rabbits. All specimens were obtained from the ear (medial artery) except at the 26th-week bleeding, which was obtained by cardiac puncture. Animal inoculations and bleedings were performed by J. W. Clark, Jr.

Fractionation The globulin fraction of the pooled sera was extracted by the half-saturated ammonium sulphate method. Protein concentration was determined

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with a Hatachi refractometer (Scientific Products) and adjusted to 1·0 g. per cent. (Cherry, Goldman, and Carski, 1960). All globulin solutions to be labelled were tested by an immunoelectrophoresis technique (LKB 6800, Sweden) to determine if albumin was present.

Conjugation The globulin fraction (protein content 1 g. per cent.) was labelled in 10-ml. aliquots (Cherry and others, 1960). Fluorescein isothiocyanate (FITC) (Baltimore Biological Laboratory) dry powder (Isomer I, chromatographically pure) was added in a dye: protein ratio of 1:40 (0·025 mg. FITC/mg. protein) (Griffin, Carski, and Warner, 1961). The solution was placed in a rotating ice bath and refrigerated for 18–20 hours. The fluorescein-labelled globulin was then dialysed for 4 hours against phosphate buffered saline (PBS) containing Dowex (J. T. Baker Chemical Company) ion resin exchange. To ensure complete removal of unbound dye, the conjugated globulin was passed through a column of G-25 Sephadex (Goldstein, Slizys, and Chase, 1961) and eluted with PBS. This process resulted in an approximate 3-fold dilution. The conjugated globulin was again adjusted to 1 g. per cent. protein.

Absorption Conjugated globulin from each bleeding was divided into four aliquots for absorption. One aliquot was not absorbed (U), one was absorbed with tissue powder only (T), the third with Reiter organisms only (R), and the final aliquot was absorbed first with tissue powder and then with Reiter organisms (TR). Tissue absorptions (U.S. Department of Health, Education, and Welfare, 1964) were carried out (100 mg. powder/ml. antibody solution) at 5°C. overnight on a rotator by using dry spleen marrow powder (Nutritional Biochemicals Corporations) which was washed three times in PBS and dried with acetone.

"Washed" Reiter treponemes for absorption, prepared by washing intact Reiter treponemes (6-day cultures) three times with sterile saline and packing by centrifugation, were added in a ratio of two parts conjugate to one part packed Reiter organisms. The solution was incubated for 3 hours at 37°C., centrifuged at high speed for 30 minutes, and stored at −20°C.

Experimental Procedure

Inhibition Technique The one-step inhibition test (Cherry and others, 1960) was performed on the conjugates absorbed with tissue powder and Reiter (TR) as a test of specific staining ability. The conjugates (TR) at each bleeding were tested with the unlabelled pre-inoculated (normal) rabbit serum, as well as their respective unlabelled immunized serum pool.

Treponema pallidum Antigens Slides were made from six lots of reconstituted, lyophilized *T. pallidum* suspensions prepared as antigens for the FTA-ABS procedure. The *T. pallida* designated as "washed" were washed several times in sterile saline and packed by high-speed centrifugation in an attempt to remove any rabbit proteins from their surface. *T. pallidum* suspensions obtained by testicular aspirations from rabbits previously infected with virulent *T. pallidum* were termed "fresh".

Cultivable Organisms Reiter organisms designated as "washed" were handled in the same manner as the "washed" *T. pallidum*. Other spirochaetal (Hanson and Cannefax, 1965) antigen slides were prepared from cultivable, nonpathogenic treponemal strains (Kazan, Kazan 2, Kazan 4, Kazan 5, Kazan 8, *Treponema skoliodonta*, Reiter, *Treponema phagedenis*, *Treponema calligyra*, English Reiter, Nichols, *Treponema minutum*, Noguchi, and *Treponema refringens*); *Treponema zuelzerae* (the free-living California mud treponeme); one strain of normal mouth flora, *Treponema microdentium*, N-39; and one borrelial strain, *Borrelia vincentii*, N-9.

Tissues These included testes, popliteal nodes, and skin from normal (non-syphilitic) and infected (syphilitic) rabbits; guinea-pig kidney infected with virulent *Leptospira*; normal and syphilitic human lymph node and skin; and lymph nodes obtained from normal and syphilitic apes. All tissues were frozen immediately after removal and stored at −20°C. until sectioned. Tissue sections (2·5 μ) were cut on a model CTR cryostat (International Equipment Co.).

Body Fluids and Pathogenic Spiral Organisms Five aqueous humour and cerebrospinal fluid slides from normal (non-syphilitic) apes were also studied. Three virulent leptospiral strains (*Leptospira pamona*, Pamona; *Leptospira bataviae*, Van Tienan; and *Leptospira canicola*, Honel Utrecht) and one virulent borrelial strain (*Borrelia hispanica*) were used as the treponemes pathogenic to man other than *T. pallidum* and were supplied by Mrs M. M. Galton. "Fresh" *Treponema cuniculi* slides were obtained by testicular aspirations from rabbits previously inoculated with these organisms.

The tissues, aqueous, and spinal fluids, and a group of treponemes known to be pathogenic to man were tested with only the 6-week tissue-and-Reiter-absorbed (TR) conjugate at a dilution of 1:10.

Stain Technique The antigen slides and sections were fixed in acetone for 10 minutes before staining. The conjugated antibodies were allowed to react with the antigens for the conventional 30 minutes at room temperature (23·29°C.) under the protection of a moist chamber to prevent exposure to direct light and possible drying of the specimens. The stain process was followed by two 5-minute wash periods in PBS with a distilled-water rinse between and after PBS treatments. Slides were gently blotted, mounting medium was added (9 parts glycerin to 1 part PBS), cover slips were applied, and the slides read immediately. Each conjugate was used undiluted and at dilutions of 1:10, 1:20, and 1:40 in PBS (pH 7·2).

Microscopical Observations These were made with a Leitz ultraviolet SM microscope equipped with a high-pressure mercury-arc lamp (OSRAM HBO-200). In determining fluorescent reactions, a BG-12 (3-mm.) barrier and Euphos (2·5-mm.) eyepiece filter were used for tissue readings, and a BG-12 and OG-1 (2·5-mm.) filter combination for examination of treponemal suspension slides.

Results

Before inoculation, sera from all rabbits were non-reactive in each of five serological tests. Three weeks after inoculation, two rabbits were seronegative in the TPI test. Serum from one rabbit and the serum pool were weakly reactive in the TPI test. All other tests were reactive at this time. The individual sera from all rabbits and the serum pool were reactive at the 6th-week bleeding and remained reactive at the 26th-week bleeding, except for one rabbit in which the serum showed VDRL conversion to non-reactive.

Results of immunoelectrophoresis on the precipitated, pooled globulin fractions showed albumin to be absent.

When the pre-inoculation (normal) rabbit serum was added to each of the four fluorescein-conjugated anti-*T. pallidum* conjugates (3, 6, 16, and 26 weeks) and the mixture was applied to slides of virulent *T. pallidum*, fluorescence was strong. There was no fluorescence, and thus inhibition was complete, when the anti-*T. pallidum* conjugate (TR) was added to the unlabelled anti-*T. pallidum* serum of the same bleeding and applied to identical slides containing virulent *T. pallidum* organisms. In this particular case it was found that equal volumes of conjugate and serum produced inhibition.

Table I shows the results obtained from testing the unabsorbed (U) and the tissue-and-Reiter-absorbed (TR) conjugates prepared from the pre-inoculation (normal) serum pool and the four interval bleeding pools at a 1:20 dilution, as well as undiluted, on 22 antigen slides. The results obtained with six lyophilized FTA-ABS antigens and the fresh *T. pallidum* were pooled and averaged for convenience in presentation because of similar results. The pre-inoculated normal rabbit serum (U), conjugated with FITC as described and used undiluted, stained all organisms tested in varying degrees except *T. microdentium* and "washed" Reiter, which were non-fluorescent. With the

TABLE I

STAINING OF UNABSORBED AND TISSUE-REITER ABSORBED CONJUGATES FROM PRE- AND POST-INOCULATION BLEEDINGS

Bleeding	Pre-inoculation				3rd-wk post-inoculation				6th-wk post-inoculation				16th-wk post-inoculation				26th-wk post-inoculation			
	Unabs.		Tissue × 1 Reiter × 1		Unabs.		Tissue × 1 Reiter × 1		Unabs.		Tissue × 1 Reiter × 1		Unabs.		Tissue × 1 Reiter × 1		Unabs.		Tissue × 1 Reiter × 1	
Dilution	Undil.	1:20	Undil.	1:20	Undil.	1:20	Undil.	1:20	Undil.	1:20	Undil.	1:20	Undil.	1:20	Undil.	1:20	Undil.	1:20	Undil.	1:20
T. pallidum	+	○	+	—	+	+	+	○	+	+	+	+	+	+	○	—	+	+	+	○
T. pallidum washed	—	—	—	—	+	—	—	—	+	+	+	+	+	○	○	—	+	—	+	—
T. cuniculi fresh	+	—	○	—	+	○	○	—	+	+	+	○	+	+	+	—	+	+	+	○
Reiter washed	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
T. zuelzerae	+	—	—	—	—	—	—	—	○	—	—	—	—	—	—	—	—	—	—	—
Skoliodonta *B. vincentii*	—	—	—	—	○	—	—	—	○	—	—	—	○	—	—	—	○	—	—	—
Phagedenis *T. refringens*	○	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	○	—	—	—
Kazan Kazan 2 Kazan 4 Kazan 5 Kazan 8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	○	—	—	—
Noguchi Minutum Nichols Calligyra Reiter Microdentium English Reiter	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

\+ Strong fluorescence ○ Moderate fluorescence — Trace or no fluorescence

conjugate at a 1:10 dilution, fluorescence was observed in all slides except cultivable Noguchi, *T. microdentium*, and "washed" Reiter, which were non-fluorescent. When the conjugate was diluted at 1:20, the lyophilized FTA-ABS antigens and the fresh *T. pallidum* demonstrated varying amounts of strong fluorescence, while "washed" *T. pallidum*, Kazan, *T. skoliodonta*, *T. phagedenis*, *T. zuelzerae*, Noguchi, *T. microdentium*, and "washed" Reiter were non-fluorescent. The remaining virulent and cultivable treponemes demonstrated variable traces of fluorescence. At the 1:40 dilution, slight fluorescence was noted in three of the FTA-ABS antigen slides and the "fresh" *T. pallidum*, Kazan 2, Nichols, *T. refringens*, and "fresh" *T. cuniculi*. All other organisms tested were non-fluorescent. It was not possible in this study to examine treponemes from yaws and pinta patients.

Because conjugates are sometimes used for demonstrating *T. pallidum* in tissue sections (Yobs and others, 1964), and because for such uses conjugates are usually absorbed with tissue powders, it was considered important to ascertain what effect absorption with tissue powder had on recognition of treponemes. Absorption with tissue powder (T) had little effect on the overall staining pattern of the organisms tested. However, when the organisms were subjected to the Reiter-absorbed (R) conjugate, fluorescence was noticeably decreased in three virulent strains of *T. pallidum* and *T. cuniculi*, but absent in all other organisms by the 1:20 dilution. At a 1:40 dilution, only slight fluorescence was observed in two of the FTA-ABS antigen slides and with the fresh *T. pallidum* and *T. cuniculi* suspensions. All other slides were non-fluorescent. Undiluted or at any dilution, the tissue-and-Reiter-absorbed (TR) conjugate failed to stain any of the avirulent treponemes and, at dilutions of 1:10 or higher, stained only four of the virulent *T. pallidum* antigens. With the conjugate at the 1:20 and 1:40 dilutions, fresh *T. pallidum* and *T. cuniculi* were barely fluorescent with poorly defined morphology.

All organisms except *T. skoliodonta*, *B. vincentii*, and the virulent *T. pallidum* and *T. cuniculi* demonstrated varying degrees of slight fluorescence and atypical morphology when the unabsorbed (U), undiluted conjugate prepared from the 3-week post-inoculation antisera was used. A 1:10 dilution completely removed fluorescence from Nichols, *T. microdentium*, and "washed" Reiter. The virulent *T. pallidum* and *T. cuniculi* demonstrated strong and good fluorescence, respectively, and all other organisms tested showed traces of fluorescence. Reactivity was eliminated by the 1:40 dilution in all cases except *T. cuniculi* which stained similarly to virulent *T. pallidum* (minimal fluorescence).

Absorption with Reiter (R) removed all staining ability for any treponeme other than virulent *T. pallidum*, *T. cuniculi*, *T. skoliodonta*, and *B. vincentii* in the undiluted conjugate. At a dilution of 1:10 or higher, only *T. pallidum* stained. Undiluted conjugate absorbed with tissue and Reiter (TR) failed to stain any of the organisms tested except virulent *T. pallidum* (strong) and *T. cuniculi* (moderate). At dilutions of 1:20 and 1:40 only virulent *T. pallidum* stained.

The ability of the conjugate to stain all avirulent organisms could be removed from the 6-week immune serum either by absorbing with Reiter (R) or by combining tissue and Reiter (TR) with no effect on the brightness of the virulent *T. pallidum*. *T. cuniculi*, although on a close parallel with *T. pallidum*, appeared consistently less fluorescent.

The undiluted, unabsorbed 16-week conjugate stained all organisms to some degree except *T. phagedenis* and cultivable Nichols; however, only the *T. pallidum*, *T. cuniculi*, *T. skoliodonta*, and *B. vincentii* stained strongly or moderately. The virulent treponemes remained fluorescent at the 1:20 dilution, but all other slides failed to demonstrate fluorescent organisms. When the conjugate was absorbed with either Reiter or a combination of tissue and Reiter and used at the same dilution (1:20), none of the tested antigens fluoresced significantly.

The "common" antibody titre definitely increased by the 26th-week bleeding. Individual avirulent treponemes appeared more fluorescent, and absorptions as described were not as equally effective. The tissue-absorbed conjugate was able to demonstrate slight fluorescence in seven of the avirulent treponemes at the 1:40 dilution, although the virulent *T. pallidum* and *T. cuniculi* remained strongly fluorescent. Reiter absorption removed all staining ability from the avirulent treponemes by the 1:40 dilution and moderately reduced the brightness of the virulent treponemes. The tissue-and-Reiter-absorbed (TR) conjugate removed all staining from avirulent treponemes, and progressively lower fluorescence was observed in the virulent treponemes until they were only slightly fluorescent at the 1:40 dilution.

The 6-week post-inoculation conjugate absorbed with tissue and Reiter (TR) and diluted 1:10 was applied to sections of normal and syphilitic rabbit testicle, lymph node, and skin; normal and syphilitic human lymph node and skin; normal and syphilitic lymph node, aqueous, and cerebrospinal fluids obtained from apes; guinea-pig kidney infected

with virulent *Leptospira*; and four spirochaetes pathogenic to man (*L. pamona*, *L. bataviae*, *L. canicola*, and *B. hispanica*). The results are shown in Table II. All infected tissues demonstrated strongly fluorescent well-defined organisms, morphologically indistinguishable from *T. pallidum*. Normal tissues, body fluids, and pathogenic spirochaetes (other than *T. pallidum*) failed to demonstrate fluorescence, although in the last, it was clearly noted when observed with tungsten light.

TABLE II

DIRECT STAIN OF TISSUES AND SPIROCHAETAL SUSPENSIONS WITH 6th-WEEK "TR" CONJUGATE AT 1:10 DILUTION

Material Tested		Fluorescent Reaction
Normal (non-syphilitic) tissues	Rabbit testicle	−
	Rabbit lymph node	−
	Rabbit skin	−
	Human lymph node	−
	Human skin	−
	Ape lymph node	−
Normal (non-syphilitic) fluids	Ape aqueous fluid	−
	Ape cerebrospinal fluid	−
Infected (leptospiral) tissue	Guinea-pig kidney	−
Infected (syphilitic) tissues	Rabbit testicle	+
	Rabbit lymph node	+
	Rabbit skin	+
	Human lymph node	+
	Human skin	+
	Ape lymph node	+
Pathogenic spirochaetal suspensions (other than *T. pallidum*)	*L. pamona*	−
	L. bataviae	−
	L. canicola	−
	B. hispanica	−

\+ Strong fluorescence with clear morphological characteristics.
− No fluorescence observed.
"TR" denotes that the conjugate was absorbed with tissue powder and Reiter treponemes.

Discussion

When applied to both virulent and cultivable avirulent treponemes, unabsorbed normal rabbit serum globulin conjugated with FITC stained all tested organisms to some degree, either undiluted or at one of the dilutions used, except *T. microdentium* and "washed" Reiter. The broad spectrum of this reactivity is consistent with the reports that many treponemes share a common antigenic factor (Deacon and Hunter, 1962; Meyer and Hunter, 1967). However, this finding extends those reports in that it shows that even pre-immune "normal" rabbit globulin used as a direct fluorescent antibody reagent possesses broad reactivity with treponemes. Part of this phenomenon may be caused by rabbit serum proteins from the culture medium absorbed on the surface of the treponemes (Gelperin, 1951).

This finding illustrates the importance of including pre-inoculation globulin as a control reagent when direct fluorescent antibody techniques are used to investigate antigenic relationships of bacteria. Some of the reactivity seen in a hyperimmune serum may result from causes other than the experimental inoculations. Although the labelling of normal serum has been mentioned in several previous studies (Coons and Kaplan, 1950; Goldstein and others, 1961; Riggs, Seiwald, Burckhalter, Downs, and Metcalf, 1958), it appears more desirable that this normal globulin should be derived from a pooled sample of sera taken from each of the animals used for the production of the final antiserum which is to be labelled with fluorescein isothiocyanate (Cherry and others, 1960).

The specific treponemal (*T. pallidum*) antibody titre was the first to rise, as shown by the maximum fluorescence demonstrated by virulent *T. pallidum* as opposed to moderate or traces of fluorescence demonstrated in any other organism at the 3rd-week bleeding (Table I). Absorption and dilution reduced the fluorescence of virulent *T. pallidum*, but in most instances fluorescence remained definitive, although the avirulent strains failed to fluoresce when the conjugated globulin was absorbed with either Reiter (R) or tissue and Reiter (TR).

By the 6th-week post-immunization bleeding, the specific (*T. pallidum*) antibody titre appeared to be at a peak, as shown by the ability of virulent *T. pallidum* to fluoresce strongly after both absorption (TR) and dilution (1:20). Group staining appeared to follow the same general pattern as the 3rd-week post-immunization antisera, with some organisms showing a slight increase in titre. Absorption of the conjugate with Reiter (R) and with tissue and Reiter (TR) removed fluorescence from all organisms other than *T. pallidum* and *T. cuniculi*, the latter remaining somewhat less fluorescent than the former. Sixteen weeks after inoculation, the conjugated antisera began to show a decline in the amount of specific (*T. pallidum*) antibody. The fluorescence was somewhat lower in the lyophilized and "washed" *T. pallidum*, as compared with the "fresh" suspensions of *T. pallidum* and *T. cuniculi*. All cultivable treponemes, except Nichols, *T. phagedenis*, and "washed" Reiter, showed some degree of fluorescence, although morphology was distinct only in *B. vincentii* and *T. skoliodonta*. A dilution of 1:20 was adequate to remove all group reactions tested, a result showing that the common

titre, although present, had not increased appreciably. Although the fluorescence of the virulent treponemes was reduced, in most cases it remained significantly higher even after absorption.

There is strong evidence that group antibodies were still being produced at the time of the 26th-week bleeding. In some cases, there appeared to be an increase in the titre of these antibodies as shown by the increased amounts of fluorescence detected. Whether this rise in titre was caused by group antibodies, unrelated antibody(ies), or a change in the type of antibody being produced was not ascertained. Absorptions with tissue powder, with Reiter organisms, or with a combination were not as effective as they were in earlier bleedings, a result indicating that the antibody content of the globulin was now greater than the absorptive ability of the materials at the ratio used. Since absorption with Reiter organisms removed all but slight amounts of fluorescence in the virulent treponemes by the 1:40 dilution, it was apparent that specific antibodies (*T. pallidum*) were not being produced in the same quantities as earlier.

An interesting feature was that syphilitic infection did not seem to provoke a rise in titre of the "common" antibody (Deacon and Hunter, 1962), as judged by the observation that the conjugates showed little if any reactivity with "washed" Reiter organisms as opposed to the virulent *T. pallidum*. This finding is offered with the caution that the "washing" process might in some way have diminished the reactivity of antigens on the Reiter organisms. Nevertheless, the "washed" Reiter organisms can be used to remove the conjugate's ability to stain the "group" antigens of treponemes; thus presumably the "group" antigen of the Reiter is intact. On the other hand, there is also the intriguing possibility that the removal of "group" reactivity from the conjugate by absorption with Reiter organisms occurs because of some mechanism other than antigen-antibody interaction.

The pattern of reactivity shown by this study helps define more precisely the conditions by which a fluorescein-conjugated antiserum may be made more specific for *T. pallidum*. This better defined fluorescent antibody reagent may prove of value not only in identifying *T. pallidum* in material from primary and secondary syphilis lesions, but also in the current clinical investigations of spiral organisms in eye and spinal fluids (Goldman and Girard, 1967; Smith and Israel, 1967a, b; Wells and Smith, 1967).

The preparation of the reagent described is discussed only in relation to its application in the conventional 30-minute stain technique; we have no information on its use in the shorter RIS technique (Kellogg and Deacon, 1964). This reagent also differs from the fluorescent antibody darkfield reagent (Kellogg and Deacon, 1965) in that it is prepared from rabbit rather than from human anti-*T. pallidum* globulins.

Summary

A fluorescein-conjugated anti-*T. pallidum* globulin (rabbit origin) for use in the direct immunofluorescent identification of *T. pallidum* in body fluids and tissue sections has been prepared. Sequential bleedings were used to ascertain the optimal time (6 weeks) for the harvesting of the antiserum, and techniques of absorption and dilutions were used to narrow its specificity.

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REFERENCES

Cherry, W. B., Goldman, M., and Carski, T. R. (1960). "Fluorescent Antibody Techniques in the Diagnosis of Communicable Diseases". Communicable Disease Center, U.S. Department of Health, Education, and Welfare. P.H.S. Publication No. 729. U.S. Government Printing Office, Washington, D.C.

Coons, A. H., and Kaplan, M. H. (1950). *J. exp. Med.*, **91**, 1.

Deacon, W. E., and Hunter, E. F. (1962). *Proc. Soc. exp. Biol. (N.Y.)*, **110**, 352.

Gelperin, A. (1951). *Amer. J. Syph.*, **35**, 1.

Goldman, J. N., and Girard, K. F. (1967). *Arch. Ophthal.*, **78**, 47.

Goldstein, G., Slizys, I. S., and Chase, M. W. (1961). *J. exp. Med.*, **114**, 89.

Griffin, C. W., Carski, T. R., and Warner, G. S. (1961). *J. Bact.*, **82**, 534.

Hanson, A. W., and Cannefax, G. R. (1965). *Brit. J. vener. Dis.*, **41**, 163.

Kellogg, D. S., Jr., and Deacon, W. E. (1964). *Proc. Soc. exp. Biol. (N.Y.)*, **115**, 963.

—— —— (1965). *Bacteriol. Proc., Amer. Soc. Microbiol.*, Abstracts of 65th Annual Meeting, p. 60, No. **M128.**

"LKB 6800 A Immunoelectrophoresis Equipment Operating Manual". LKB Prodokter AB, P.O. Box 12220, Stockholm 12, Sweden.

Meyer, P. E., and Hunter, E. F. (1967). *J. Bact.*, **93**, 784.

Mothershed, S. M., Yobs, A. R., and Clark, J. W., Jr. (1967). *Brit. J. vener. Dis.*, **43**, 267.

VDRL "Provisional Technique for the Fluorescent Treponemal Antibody-Absorption (FTA-ABS) Test," September 20, 1965, available on request from the Venereal Disease Research Laboratory, National Communicable Disease Center, Atlanta, Georgia 30333.

Riggs, J. L., Seiwald, R. J., Burckhalter, J. H., Downs, C. M., and Metcalf, T. G. (1958). *Amer. J. Path.*, **34**, 1081.

Smith, J. L., and Israel, C. W. (1967a). *Arch. Ophthal. (Chicago)*, **77**, 474.

Smith, J. L., and Israel, C. W. (1967b). *J. Amer. med. Ass.*, **199**, 980.
U.S. Department of Health, Education, and Welfare (1964), "Serologic Tests for Syphilis". P.H.S. Publication No. 411. U.S. Government Printing Office, Washington, D.C.
Wells, J. A., and Smith, J. L. (1967). *Arch. Ophthal. (Chicago)*, **77**, 530.
Yobs, A. R., Brown, L., and Hunter, E. F. (1964). *Arch. Path.*, **77**, 220.
—— Clark, J. W., Jr., Mothershed, S. M., Bullard, J. C., and Artley, C. W. (1968). *Brit. J. vener. Dis.*, **44**, 116.
—— Olansky, S., Rockwell, D. H., and Clark, J. W., Jr. (1965). *Arch. Derm. (Chicago)*, **91**, 379.
—— Rockwell, D. H., and Clark, J. W., Jr. (1964). *Brit. J. vener. Dis.*, **40**, 248.

La préparation de la globuline immuno-fluorescente pour l'identification du *Treponema pallidum*

RÉSUMÉ

Une préparation de l'anti *T. pallidum* globuline conjugée à la fluorescine obtenue du lapin afin de servir à l'identification par immuno-fluorescence directe du *T. pallidum* dans les liquides du corps et dans les sections de tissues a été composée. Des prises de sang faites subséquemment ont été faites afin d'établir le temps optimum (6 semaines) pour la récolte de l'antisérum, et les techniques d'absorption et de dilution ont été employées pour restreindre sa spécificité.